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Murine cytomegalovirus-inhibitory effects of ImuVert

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Summary

ImuVert, a sterile preparation composed primarily of *Serratia marcescens* membrane vesicles and ribosomes, was significantly inhibitory to murine cytomegalovirus (MCMV) infections in BALB/c mice. Antiviral activity was manifested as increased survivor number and decreased recoverable virus titers in spleens, lungs and salivary glands. Treatments were intraperitoneal (i.p.) beginning 24 h pre, 4 h post- or 24 h post-virus inoculation and then repeated 4 days later. Doses of 5, 16 or 50 μg/mouse were effective; 160 μg/mouse, which caused host weight loss in toxicity controls, was not inhibitory to the infection. A single i.p. treatment of mice substantially augmented natural killer (NK) cell activity and increased total B-cells, while reducing total T- and T-helper cells. A late (48 h) decline in T-cell function and transient increases in B-cell function were observed in the treated animals. Serum interferon was not induced. Mice pretreated with anti-asialo GM1 antibody to reduce their NK cell populations, then infected with MCMV and treated with ImuVert were protected to the same degree as normal animals. Severe combined immunodeficient mice infected with MCMV and treated with ImuVert were not protected from the infection. These data suggest ImuVert to act by a mechanism other than NK cell activation in preventing MCMV infections.

ImuVert; Cytomegalovirus; Antiviral; Natural killer cell; Immunomodulator

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Introduction

The biological response modifier ImuVert™ is a sterile preparation from the bacterium *Serratia marcescens*. It consists of sized vesicles derived from the bacterial membrane, associated with ribosomes and suspended in a Tris/magnesium buffer (Cell Technology, Investigational Brochure, March, 1991). The crude *S. marcescens* extracts were first described by Urban et al. (1980) to have tumor immunotherapeutic activity, the tumor inhibited being cutaneous SaD2 fibrosarcomas induced in DBA/2 mice. Later studies by Jimenez et al. (1990) also indicated ImuVert to protect against chloroleukemia in rats. The material has been under phase II clinical evaluation against certain forms of cancer, particularly recurrent malignant astrocytomas (Jaeckle et al., 1990). ImuVert has been shown to stimulate natural killer (NK) cell activity, antibody-dependent cellular cytotoxicity (ADCC) and cytotoxic T-cell activity in treated peripheral blood mononuclear cells from healthy human volunteers (Warren et al., 1989a, 1989b). A stimulation of differentiation of monocytes has also been reported (Jimenez et al., 1990).

The stimulation of NK cells and ADCC by ImuVert parallels the similar immunomodulation reported for 7-thia-8-oxoguanosine (Smee et al., 1990) and for 2-amino-5-bromo-6-phenyl-4(3H)pyrimidinone (ABPP) and 2-amino-5-iodo-6-phenyl-4(3H)pyrimidinone (AIPP) (Lotzova et al., 1983; Wierenga, 1985). These immunomodulators have been shown to significantly inhibit experimentally-induced murine cytomegalovirus (MCMV) infections in mice (Smee et al., 1990; Brideau and Wolcott, 1985). These immune stimulation mechanisms have been considered to be implicated in MCMV disease inhibition and prompted studies to investigate ImuVert's efficacy against this murine infection, the human counterpart being a highly significant disease particularly of immunocompromised individuals (Rubin, 1990; Schooley, 1990). The experiments performed to investigate ImuVert's efficacy against MCMV and to determine some of its immunological effects in mice are the subject of this report.

Materials and Methods

Virus

The Smith strain of MCMV, obtained originally from the American Type Culture Collection (ATCC, Rockville, MD), was used. The virus was a salivary gland preparation titrated in mice prior to use in these studies. The Smith strain of vesicular stomatitis virus, also obtained from the ATCC, was used for interferon induction studies. A pool of this virus was prepared from infected mouse L cells.

Mice

Three week-old female specific-pathogen free BALB/c mice weighing 9–11 g

were obtained from Simonsen Laboratories (Gilroy, CA). They were maintained in a non-sterile environment. Severe combined immune-deficient (SCID) mice, congenic partners of BALB/c AnIcr mice, as initially described by Bosma and colleagues (1983) were raised in this laboratory from breeding pairs provided by Dr. Norman Klinman of Scripps Institute (La Jolla, CA). The SCID animals were housed in microisolator cages (Lab Products, Maywood, NJ) containing sterilized bedding (Pawns Bedd), food (Wayne Lab Blox) and water. The cages were held in HEPA-filtered horizontal laminar flow hoods (Lab Products). The SCID mouse colony was managed in a manner to keep the incidence of leaky (immunoglobulin-producing) phenotype to less than 5% (Bosma, 1989)

Compound

Freshly prepared ImuVert was provided by Cell Technology and was used within 7 days of receipt. The material was kept frozen at −20°C until used. Immediately prior to injection, the ImuVert was dissolved in a buffer solution provided by Cell Technology. Experiments using immunosuppressed mice utilized a new, lyophilized ImuVert preparation that was stored at 4°C until used. Data provided by Cell Technology indicated the lyophilized material had the same potency and a longer shelf-life than the freshly prepared material. Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine], used as a positive control (Freitas et al., 1985), was obtained from Dr. Elmer Reist of SRI International (Menlo Park, CA). This material was dissolved in physiological saline for these studies.

Assay for tissue virus

All tissues to be assayed were homogenized to a 10% (w/v) suspension in minimum essential medium containing 2% fetal bovine serum, bicarbonate buffer and gentamicin. Each sample was diluted though a series of 10-fold dilutions and each assayed in triplicate in mouse 3T3 cells, with viral cytopathic effect (CPE) endpoint read microscopically after a 6-day incubation at 37°C. The method of Reed and Muench (1938) was used to calculate virus titers, which were expressed in $\log_{10}$ cell culture infectious doses per ml.

Splenic cell subpopulations

Subpopulations of splenic cells from treated, uninfected mice were enumerated with a fluorescence-activated cell sorter (FACS) (EPICS-C, Coulter, Hialeah, FL). Dispersed splenocytes were reacted with fluorosceinlabeled isothiocyanate- or phycoerythrin-labeled murine antibodies (Becton-Dickinson, Cockeysville, MD) as follows: anti-thy 1.2 (total T-cells); anti-L3T4 (T helper cells); anti-Lyt 2 (T suppressor/cytotoxic cells); and anti-Ly5 (total B-cells).

Natural killer (NK) cell activity

Splenic cells from treated, uninfected mice were assayed for their ability to

lyse YAC-1 tumor cells in a conventional 4 h chromium release assay as an indicator of NK cell function (Warren et al., 1985). Ratios of splenic cells to tumor cells used were 5:1, 25:1, 50:1 and 100:1. Cytotoxicity was expressed as: % chromium release = [(experimental counts per minute [CPM] – background CPM)/(maximum CPM – background CPM)] × 100.

T- and B-cell blastogenesis assays

Blastogenesis assays were performed by adding 1×10^5 spleen cells from treated, uninfected mice to triplicate wells of flat-bottomed 96-well microplates in a volume of 0.1 ml. Concanavalin A (Con A) at concentrations of 0.5, 1 or 2 μg/ml or lipopolysaccharide (LPS) at 2.5, 5 or 10 μg/ml were added to each well in 0.1 ml aliquots. During the last 24 h of a 48 h incubation at 37°C, the cells in each well were pulsed with 0.4 μCi of [^{3}H]thymidine (New England Nuclear, Boston, MA). The cells were harvested on glass fiber paper and the uptake of radioactivity determined. The proliferative responses were expressed as CPM of [^{3}H]thymidine incorporation.

Interferon (IFN) assay

Sera from treated, uninfected mice were placed in 0.1 ml amounts on an 18–24 h monolayer of mouse L cells in flat-bottomed 96-well microplates and incubated 24 h at 37°C. Growth medium was drained from the plates and 0.1 ml of 10^3 50% cell culture infectious doses of vesicular stomatitis virus, strain Indiana, was added to each well and the plates incubated 6 days at 37°C. Viral CPE was then determined microscopically. Controls included cells exposed to test medium and then to virus, cells exposed to test medium only, and a positive control consisting of cell culture medium containing IFN of known titer which had been adjusted to international units. IFN titer was defined as the reciprocal of the lowest dilution inhibiting viral cytopathic effect by 50%.

Experiment design – immunocompetent animal studies

An experiment was initially performed to determine ImuVert's effects on NK cell activity, T- and B-cell function, splenic T-, T-helper, T-suppressor and B-cell enumeration, and IFN induction in mice. Groups of nine mice were each injected intraperitoneally (i.p.) with 5, 16 or 50 μg of ImuVert/mouse. Groups of three animals at each dosage level were killed 4, 24 and 48 h later, and the blood and spleens taken for the above immunologic assays. The same tissues were also taken from normal untreated animals as controls. Sera was frozen at −85°C for later interferon assay. The assays using spleens were done immediately after collection of the tissues. As will be shown in Results, these data showed immunostimulatory effects lasting at least 48 h, which indicated repeated treatments with biological response modifiers should be spaced 3 to 4 days apart. This was done in the following antiviral experiments.

A series of experiments were performed to evaluate the murine MCMV-inhibitory effects of ImuVert. In all experiments with BALB/c mice, the animals were infected i.p. with a 10^6 cell culture 50% infectious dose ($CCID_{50}$)

of virus. SCID mice received 10^4 $CCID_{50}$ of virus. These virus concentrations were lethal to approx. 90% of the respective animal strains. In all experiments, 5 sham-infected mice were treated with each dosage of drug as toxicity controls, and 5 uninfected, untreated mice were held as normal controls. These control animals were weighed prior to initial treatment and again 18 h after the final treatment to determine effects of therapy on host weight gain.

In the first experiment, ImuVert at doses of 5, 16, 50 and 160 μg/mouse was injected into 10 MCMV-infected mice/dose, with 20 infected, placebo-treated animals run as controls. Treatments were 24 h pre-virus inoculation and again 96 h later. Ganciclovir at 50 mg/kg/day was given to 10 MCMV-infected mice i.p. twice daily for 5 days beginning 4 h post-virus inoculation as positive control. The animals were observed daily for death for 21 days.

The above ImuVert treatment schedule was repeated in a second experiment, with the material at 5, 16 and 50 μg/mouse administered to 28 mice per drug dosage. Thirty infected mice were concomitantly treated with buffer as virus controls. On days 2, 4, 6, 9, 12 and 21 post-virus inoculation, 3 mice in each group were randomly selected, bled, killed and their spleens, lungs, livers, kidneys and salivary glands removed and assayed for virus titer.

A final experiment utilized delayed therapy; ImuVert at 5, 16 and 50 μg/mouse was injected i.p. 4 h and 4 days or 24 h and 5 days post-virus inoculation, with tissue virus determinations done on day 6, only.

Experiment design – immunosuppressed animal studies

To determine if NK cells played a role in ImuVert's anti-MCMV activity, 30 BALB/c mice were injected i.p. with 50 μl of undilute anti-Asialo GM1 (anti-NK cell) antibody (Wako BioProducts, Richmond, VA). One day later, 15 of these animals and 15 normal mice were treated i.p. with ImuVert (50 μg/mouse). The other 15 antibody-treated mice and 15 additional normal mice were concomitantly injected i.p. with placebo. Five animals in each group were killed 24 h later and their spleens removed and assayed for NK cell activity. All remaining animals were infected with MCMV and observed daily though 21 days for incidence of death. Those originally receiving ImuVert were treated a second time 72 h after virus inoculation; placebo was again administered to the originally placebo-treated animals.

One experiment was run to determine ImuVert's efficacy in MCMV-infected SCID mice. The material at dosage levels of 20 and 60 μg/mouse was administered i.p. 24 h pre-MCMV injection and then on days 3, 7, 11, 15 and 19 post-virus exposure. Placebo was concomitantly administered to a separate group of mice. Ten animals were used in each group. All were observed through day 30 for death.

Statistical analyses

Increases in survivors compared with virus controls were evaluated using chi-square analysis with Yates' correction. The *t*-test was employed to analyze differences in mean survival times and decreases in virus titers in tissues.

Differences in means in the NK cell, T cell function and B cell function assays were analyzed using a split plot analysis of variance (ANOVA); flow cytometer values were evaluated using a 2-way factorial ANOVA program.

Results

Effects of therapy 24 h pre- and 72 h post-virus inoculation in immunocompetent mice

Treatment with ImuVert in doses of 5 to 50 μg/mouse resulted in a significant increase in surviving MCMV-infected mice (Table 1). Ganciclovir treatment was also effective, preventing deaths in all infected mice. The 160 μg/mouse dose of ImuVert resulted in a slight host weight loss in the toxicity control animals and no discernible antiviral effect. The animals gained weight at all other dose levels, although to a lesser extent than either normal controls or ganciclovir-treated controls.

Significant virus titers were seen in lungs, spleens and salivary glands but not in livers or kidneys of placebo-treated mice. The liver homogenates were somewhat cytotoxic, masking any virus which may have been present. ImuVert therapy markedly reduced the amount of virus recoverable from the spleen (Fig. 1B), and lowered the lung virus titers to varying degrees depending on dosage used (Fig. 1A). The virus titers in the salivary glands were reduced less than one $\log_{10}$ at all dosages (Fig. 1C). No survivor data were obtained in these virus titer experiments.

Effects of delayed treatment initiation in immunocompetent animals

When ImuVert treatment was delayed to begin 4 h post-virus inoculation and then repeated 4 days later, an inhibition of the MCMV infection still

TABLE 1

Effect of ImuVert therapy[a] on lethal murine cytomegalovirus infections in mice

Compound	Dosage (μg/mouse)	Tox. Control		Infected, Treated	
		Surv/Total	Host Wt. Change (g)[b]	Surv/Total	MST[c] (days)
ImuVert	160	5/5	−0.2	2/10	6.9 (2.2)
	50	5/5	0.9	8/10**	7.0 (1.4)
	16	5/5	0.7	5/10*	8.6 (1.7)
	5	5/5	1.5	6/10*	7.8 (0.5)
Ganciclovir	50 mg/kg/day	5/5	1.7	10/10**	>21.0 (0.0)**
Buffer	–	–	–	3/20	7.1 (0.7)
Normals	–	5/5	2.1	–	–

[a]i.p. injections 24 h pre- and 72 h post-virus inoculation.

[b]Difference between initial weight at start of treatment and weight 18 h following final treatment of toxicity control mice.

[c]Mean survival time of mice dying on or before day 21 (one standard deviation).

*$P < 0.05$, **$P < 0.01$.

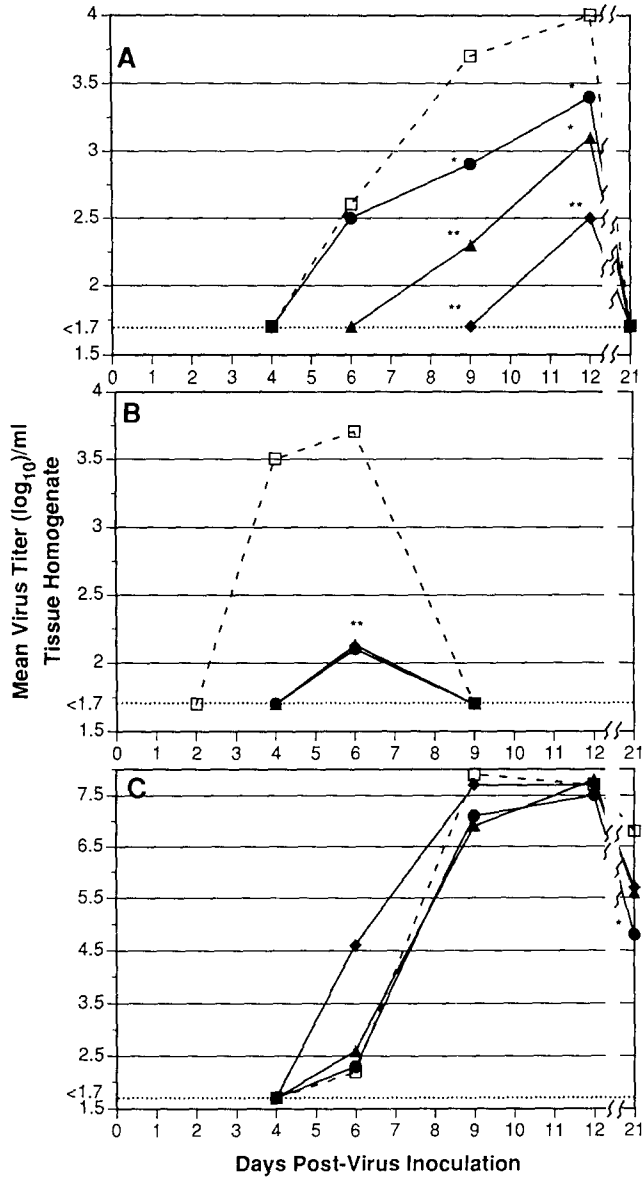

Fig. 1. Effect of i.p. ImuVert treatment on MCMV development in tissues of BALB/c mice. Data expressed as geometric means. A: Lungs; B: Spleens; C: Salivary glands. □ Placebo; ● 50 μg/mouse; ▲ 16 μg/mouse; ◆ 5 μg/mouse. *P <0.05, **P <0.01 compared to place

occurred as seen by lowered virus titers in lungs, spleens and salivary glands (Table 2) but this effect was generally less pronounced than that observed using the earlier therapy initiation.

When treatment was delayed to begin 1 day and 5 days after virus

TABLE 2

Effect of delayed ImuVert treatment on sub-lethal cytomegalovirus infections in mice

Compound	Dosage (μg/mouse)	Tox. Control		Infected, Treated 4 h, 4 days post-virus inoculation			Infected, Treated 24 h, 5 days post-virus inoculation		
		Surv/ Total	Host Wt. Change (g)[a]	Lung Virus Titer[b]	Spleen Virus Titer	Salivary Gland Virus Titer	Lung Virus Titer	Spleen Virus Titer	Salivary Gland Virus Titer
ImuVert	50	5/5	1.0	2.6 (1.0)	5.6 (0.5)	<1.7 (0.0)*	2.6 (1.0)	<1.7 (0.0)**	2.8 (1.5)
	16	5/5	2.0	2.7 (0.9)	5.0 (0.3)	<1.7 (0.0)*	2.6 (0.9)	4.0 (1.8)	3.0 (1.7)
	5	5/5	2.8	<1.7 (0.0)*	3.2 (2.0)	<1.7 (0.0)*	2.3 (0.7)	3.4 (1.3)	2.8 (1.4)
Buffer	–	–	–	3.1 (1.0)	4.2 (0.5)	3.2 (1.1)	3.1 (1.0)	4.2 (0.5)	3.2 (1.1)
Normals	–	5/5	3.3	–	–	–	–	–	–

[a]Difference between initial weight at start of treatment and weight 18 h following final treatment of toxicity control mice.
[b]Log_{10} geometric mean/ml of homogenates of tissues taken on Day 6 (one standard deviation).
*P <0.05, **P <0.01.

inoculation, modest virus titer reductions occurred in lung and salivary gland tissues. A significant reduction was seen only in spleens from mice receiving the highest ImuVert dosage (Table 2).

Effects on immunologic parameters in immunocompetent mice

A strong augmentation of NK cell activity was seen in uninfected mice treated with a single injection of all three doses of ImuVert (Table 3). This effect was strongly manifested 24 and 48 h after virus inoculation, but was almost inapparent 4 h after ImuVert injection, which was the earliest time the assay was done. The increased NK cell activity was seen at all effector:target cell ratios.

These same ImuVert-treated animals also exhibited significant rises in percentage of splenic B-cells, accompanied by concomitant declines in total T- and T-helper cells, all occurring to significant degrees by 24 and 48 h after treatment (Table 4). T-cell function was unaffected at 4 and 24 h but was reduced up to 80% ($P < 0.01$) in a dose-responsive fashion by 48 h after treatment (data not shown). B-cell function was only transiently affected, with significant ($P < 0.05$ or $P < 0.01$) stimulation occurring with all ImuVert doses 24 h after treatment (data not shown). IFN was not detected in the serum of treated mice at the 4, 24 and 48 h post-treatment assay times.

TABLE 3

Augmentation of natural killer cell activity in BALB/c mice by single i.p. injection of ImuVert

ImuVert Dose (μg/mouse)	Mean Release (SD)[a] at Effector:Target Cell Ratio			
	100:1	50:1	25:1	5:1
4 h post-injection				
50	6.8 (1.6)	7.4 (1.6)	4.4 (1.1)	3.2 (1.1)
16	6.4 (1.6)	7.8 (2.8)	4.7 (1.8)	5.7 (1.6)
5	5.5 (1.9)	6.4 (1.4)	5.0 (1.3)	4.6 (1.4)
0	4.8 (1.9)	5.0 (1.7)	4.2 (1.7)	3.8 (1.9)
24 h post-injection				
50	33.8 (5.3)**	28.0 (5.3)**	20.9 (1.4)**	15.7 (1.9)**
16	28.0 (5.3)**	22.6 (3.0)**	19.0 (2.2)**	15.5 (1.1)**
5	24.5 (6.3)**	23.1 (8.6)**	15.4 (3.5)**	12.4 (2.7)**
0	10.4 (2.5)	9.2 (4.0)	7.7 (2.4)	5.4 (1.9)
48 h post-infection				
50	22.2 (2.2)**	18.2 (2.3)**	12.9 (1.1)**	9.4 (1.3)**
16	18.9 (4.2)**	17.2 (4.7)**	12.4 (2.4)**	9.2 (2.1)*
5	16.3 (2.6)**	12.3 (2.7)**	9.0 (2.0)	7.6 (2.3)
0	9.0 (3.2)	7.8 (2.2)	6.4 (1.6)	5.4 (2.0)

[a]Mean percent chromium release (one standard deviation) at effector/target cell ratios indicated. $n = 3$.

*$P < 0.05$, **$P < 0.01$, compared to placebo controls run at the same time.

TABLE 4

Effect of i.p. ImuVert treatment on splenic T- and B-cell populations in BALB/c mice

ImuVert Dose (μg/mouse)	% Cells[a] (SD)			
	Total T	T-helper	T-suppressor	B
4 h post-injection				
50	53 (7)	33 (6)**	11 (2)	44 (7)**
16	53 (8)	31 (3)**	10 (1)	43 (8)**
5	53 (5)	33 (3)*	12 (1)	42 (6)**
0	56 (3)	42 (5)	13 (2)	32 (3)
24 h post-injection				
50	39 (3)**	34 (3)**	13 (4)	67 (5)**
16	39 (6)**	33 (4)**	10 (3)	62 (8)**
5	43 (5)	37 (3)	11 (2)	62 (4)**
0	47 (4)	41 (2)	13 (1)	45 (3)
48 h post-infection				
50	48 (4)*	27 (1)**	9 (1)**	52 (2)**
16	48 (4)*	27 (2)**	11 (2)**	54 (3)**
5	49 (3)	35 (3)	13 (3)	45 (4)**
0	53 (5)	41 (5)	17 (5)	30 (3)

[a]Cell numbers determined by flow cytometry. $n=3$.
*$P<0.05$, **$P<0.01$, compared to placebo controls run at the same time.

Effect of therapy in immunosuppressed animals

In order to evaluate the role of NK cells in the antiviral activity of ImuVert, mice were treated with a high dose of NK cell-destroying antibody. The results are summarized in Table 5. In normal (non-ImuVert-treated) mice receiving antibody, a reduction in mean percent chromium release occurred, depending on the effector:target cell ratio. In ImuVert-treated animals, the antibody treatment resulted in a reduction in percent chromium release at all effector:target cell ratios. ImuVert's efficacy against the MCMV infection

TABLE 5

Effect of anti-NK cell antibody[a] on NK cell activity[b] in normal and ImuVert-treated BALB/c mice

Treatment		Mean Release (SD)[c] at Effect:Target Cell Ratio			
Antibody (50 μl)	ImuVert (50 μl/mouse)	100:1	50:1	25:1	5:1
−	−	23.2 (3.1)	19.6 (5.5)	13.2 (1.5)	11.5 (1.4)
−	+	77.5 (6.0)	64.5 (11.0)	48.2 (8.0)	37.7 (7.3)
+	−	8.6 (1.8)**	5.5 (1.1)**	4.2 (0.9)**	4.4 (0.9)**
+	+	9.6 (0.9)**	7.4 (0.8)**	5.1 (0.4)**	4.3 (0.8)**

[a]Anti-asialo GM1, administered i.p. 24 h prior to ImuVert or ImuVert placebo.
[b]NK cell activity determined 24 h after ImuVert or ImuVert placebo injection.
[c]Mean percent chromium release (one standard deviation) at effector:target cell ratios indicated. $n=5$.
**$P<0.01$, compared to appropriate groups not treated with antibody.

TABLE 6

Effect of ImuVert therapy[a] on lethal murine cytomegalovirus infections in immunosuppressed mice

Host	Treatment	Surv/Total	MST[c] (days)
Normal BALB/c mice	Buffer	1/10	4.9 (0.9)
Normal BALB/c mice	ImuVert (50 μg/mouse)	2/10	6.9 (1.1)*
Antibody-treated[b] BALB/c mice	Buffer	0/10	4.3 (1.1)
Antibody-treated[b] BALB/c mice	ImuVert (50 μg/mouse)	2/10	7.8 (1.3)**
SCID mice	Buffer	2/10	21.4 (3.6)
SCID mice	ImuVert (60 μg/mouse)	0/10	20.3 (2.9)
SCID mice	ImuVert (20 μg/mouse)	0/10	20.3 (3.1)

[a]i.p. treatment 24 h pre- and 72 h post-virus inoculation.
[b]i.p. treatment with anti-Asialo GM1 antibody 48 h pre-virus inoculation.
[c]Mean survival time of mice dying on or before day 30 (one standard deviation).
*$P < 0.05$, **$P < 0.01$, compared to appropriate buffer-treated controls.

was not inhibited by this elimination of NK cell activity (Table 6); treatment with the compound resulted in significant increases in mean survival time in both normal and in antibody-treated mice. In this study, ImuVert treatments did not prevent mortality, as was observed in Table 1. This may be attributed to the more severe infection in this experiment as evidenced by the shorter time to death in the placebo group.

The SCID mice infected with a low challenge dose of MCMV died more slowly than immunocompetent mice, with a mean survival time of approx. 21 days (Table 6). Treatment with 20 or 60 μg/mouse of ImuVert had no discernible effect in preventing or delaying death in these animals.

Discussion

The biological response modifier, ImuVert, was able to elicit a significant degree of protection in immunocompetent mice against lethal challenge by MCMV. This protection was seen in the prevention of death and/or increase in mean survival time as well as inhibition of the development of viral titers in the tissues assayed. The protection was most evident when the material was administered prior to or early in the virus infection; however, even when therapy was delayed until 24 h after virus inoculation, inhibition of virus titer was still demonstrable, although to a lesser extent than that seen when ImuVert therapy was begun earlier. The antiviral effects occurred at all doses (5–50 μg/mouse) which were well tolerated in the mice, based on absence of lethality and on host weight gain in toxicity controls.

The immunological effects elicited by ImuVert therapy which have been described here suggest some possible mechanism(s) whereby this antiviral effect may have occurred. The material had no in vitro antiviral effect using inhibition of viral cytopathic effect in mouse fibroblast (3T3) cells (data not shown). The material was found to strongly stimulate NK cell activity after a

single injection in mice; this stimulatory effect parallels the augmentation of NK cell activity seen in in vitro-stimulated human peripheral blood mononuclear cells described by Warren et al. (1989). NK cell activity has been reported to be important for the control of acute MCMV disease in mice (Bancroft et al., 1981; Ebihara and Minamishima, 1984)), and has been implicated, together with cytotoxic T-cells, as important in human recovery from MCMV infection (Quinnan et al., 1981, 1982). The experiment run in NK cell-suppressed BALB/c mice was designed to illustrate the role of NK cells in this protection; it was surprising to find that ImuVert was just as efficacious in these mice as in normal animals. These results indicated the anti-MCMV effects of this immunomodulator were not due to NK cell stimulation. The anti-asialo GM1 antibody used in this study is a recognized suppressor of NK cell activity; asialo GM1, however, is also expressed on some T cells, including the precursors of cytotoxic cells and IL-2-producing cells (Pollack, 1986).

The increased numbers of B-cells associated with ImuVert treatment may aid in rendering an increased humoral immunity which also plays a role in protection against the disease (Doerr et al., 1987; Osborn et al., 1968). Since neutralizing antibody usually occurs later (14–21 days) in the disease, it would not be expected to play a major role in protection against the acute infection (Osborn et al., 1968). The late decline in T-cell function, associated with concomitant decrease in total T and T-helper cells, apparently did not appreciably influence the progress of the disease. In an unpublished study, we have found that in vitro exposure to ImuVert inhibited the proliferation of human T-cells and murine T-cell lines. The basis for this inhibitory activity is not known. It is unclear at this point whether the immune augmentation induced by ImuVert affected the MCMV disease by partially preventing the virus-induced immunosuppression or aided in improving recovery of the animals from immunosuppression.

Interferon induction was not seen in the ImuVert-treated mice, although it is possible that the 4, 24 and 48 h sampling times may have missed a sharp peak response. Chong et al. (1983) have indicated that IFN plays a role in defense against MCMV infections but Kern et al. (1978) did not find that exogenous IFN protected MCMV-infected mice. Kern et al. (1978) did find the interferon inducers poly I:C and poly ICLC to be moderately protective. The anti-MCMV disease effects of the pyrimidinones ABPP and AIPP described by Brideau and Wolcott (1985) could not be fully attributed to IFN induction, since AIPP is a poor inducer of circulating IFN relative to ABPP (Lotzova et al., 1983). In that study, ABPP was somewhat more effective against the MCMV disease than AIPP, suggesting that the IFN induction coupled with NK cell activation may be important (Brideau and Wolcott, 1985). The purine nucleoside 7-thia-8-oxoguanosine which inhibits MCMV disease (Smee et al., 1990b) also induces IFN and augments NK cell activity (Smee et al., 1990a, 1990b). These data, particularly viewed in the context of the results reported here with ImuVert, indicate that IFN induction may be one of several factors playing a role in protection against MCMV infection.

The lack of anti-MCMV activity by ImuVert in SCID mice provides further insight into the possible mechanism of action of this compound. The SCID mouse functionally is defective in its ability to respond to mitogens, to reject allogeneic grafts, to generate cytotoxic effectors, including cytotoxic T cells, and to secrete serum IgG (review, Dorshkind, 1991). The animals are lacking in T and B cells but do have functional NK cells expressing both the NK 2.1 antigen and Asialo GM1 (Dorshkind et al., 1985). ImuVert's failure to be effective in these animals would imply one or more of the above immunologic functions to be key.

A problem in the evaluation of immunomodulatory substances is the selection of the treatment regimen used in administering the materials. ImuVert's immune stimulatory effects, which were relatively slow to appear but which persisted though 48 h, prompted our spacing the administration of the material 4 days apart in an attempt to avoid stimulation during a hyporesponsive period in the animal. Another problem with studying such immunomodulators is the possible biphasic immunologic stimulation, where certain doses, such as those approaching sub-lethal toxicity, are immunosuppressive. This effect may have occurred with ImuVert in our initial study, since the highest (160 μg/mouse) dose resulted in loss of weight in toxicity controls and a lack of significant antiviral effect. It is important, however, that demonstrable disease inhibition occurred at doses considerably below that highest dose, indicating a reasonable therapeutic index for this immunomodulator.

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